What You Need To Know About Testing Sputum Samples:

Culture Identification Edition

I. What is Culture Identification?

- Culture identification occurs when a laboratory scientist detects acid fast bacilli (AFB) growth.
- High-Performance Liquid Chromatography (HPLC) and DNA Probe are tests that identify growth.
  - The DNA probe is ordered when the HPLC is indeterminate or if two organisms exist in the same culture.

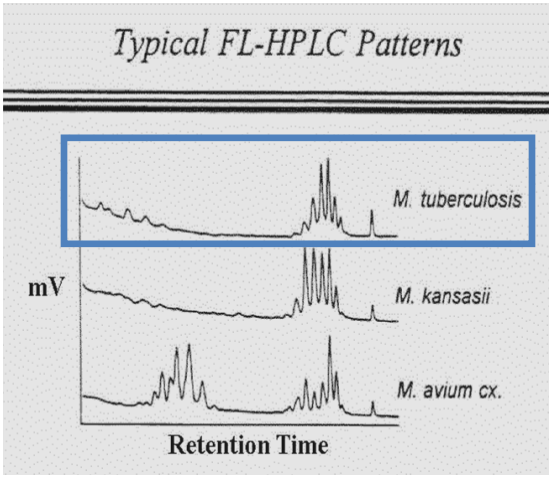
II. Why do we run these tests?

- We run these tests to identify *Mycobacterium tuberculosis* (MTB) and nontuberculosis mycobacteria (NTM).
- Culture is the gold standard for confirming active TB disease.

III. How does the laboratory run these tests?

High Performance Liquid Chromatography (HPLC):

1. Once a specimen shows acid-fast bacilli growth, the clinical scientist begins the identification process.
2. A liquid using the growth sample is prepared and placed in a tube.
3. The tube is processed by a chromatograph machine. The machine analyzes mycolic acids found in the cell walls of the bacteria.
4. Each mycobacterium has a unique amount of mycolic acid. This is read by the chromatograph machine, which produces a chromatographic pattern or "fingerprint" of the bacteria. This distinguishes TB bacteria from other kinds of mycobacteria. Results are sent to TB Control.
5. If MTB is identified, a drug susceptibility test is performed.



Chromatograph patterns for MTB and NTM

DNA Probe

1. A liquid is prepared with the growth sample in one tube using two steps:
   a. Amplification: The tube is shaken and heated to separate and multiply the nucleic acid.
   b. Detection: A light emitting mixture is added to the tube. If MTB is present, the mixture will bind to the nucleic acid and produce a unique amount of light.
2. The sample will be put into a luminometer. This will read the amount of light the tube is giving off and print results.

DNA probe is very similar to the NAAT. However, you can only run this test when AFB growth is identified.

Luminometer printing out results

IV. Results: What to Expect

- Results are usually reported to TB Control within 21 days of submitting a sputum as:
  - **HPLC**: MTB Complex, Other Mycobacteria
  - **DNA Probe:** MTB Complex, M. avium complex, M. kansasii, M. gordonae
- Programmatic decisions are made based upon the following:

Culture Results	Confirmed TB Case?	TB Contact Investigation	TB Case Management
Positive for MTB	Yes	Yes, if pulmonary	Yes
Negative for MTB	Unclear – could be a clinical case	Stop CI activities	Yes, if a clinical case
Positive for NTM	No	Stop CI activities	Refer patient to private physician for follow-up.

V. Next Steps

- It's TB! Which medication will be able to stop it? See **MTB Drug Susceptibility Testing** for more information.

Developed by Houston Department of Health and Human Services - Bureau of TB Control